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Heparan Sulfates Regulate Angiogenic Signalling and Drug Response in Aggressive Ovarian Cancer

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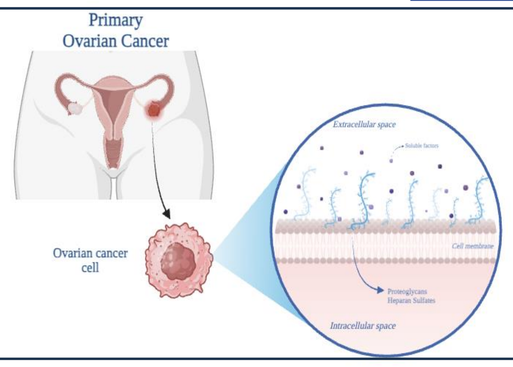
INTRODUCTION & AIM



Heparan sulfate proteoglycans (HSPGs) are essential components of the cell surface and extracellular matrix that regulate growth factor signalling, angiogenesis, and tumor progression [1]. Alterations in heparan sulfate (HS) biosynthesis have been linked to cancer development by promoting angiogenic signalling, extracellular matrix remodeling, and therapy resistance [2-3]. In ovarian cancer, EXT1, a key enzyme involved in HS chain elongation, has emerged as a critical modulator of tumor progression through the regulation of HS structure and growth factor availability within the tumor microenvironment [4-5].

This study investigates the role of EXT1-mediated HS remodeling in ovarian cancer progression, focusing on its impact on angiogenesis and platinum resistance through the spatial control of pro-angiogenic factors and drug sequestration.

METHOD

Survival analysis was performed using Kaplan–Meier plots generated with the KMplot tool *(http://kmplot.com/analysis/)*, based on TCGA ovarian cancer datasets.

Cell model: PEO1 cells were transfected with an EXT1 overexpression plasmid and subsequently selected using G418.

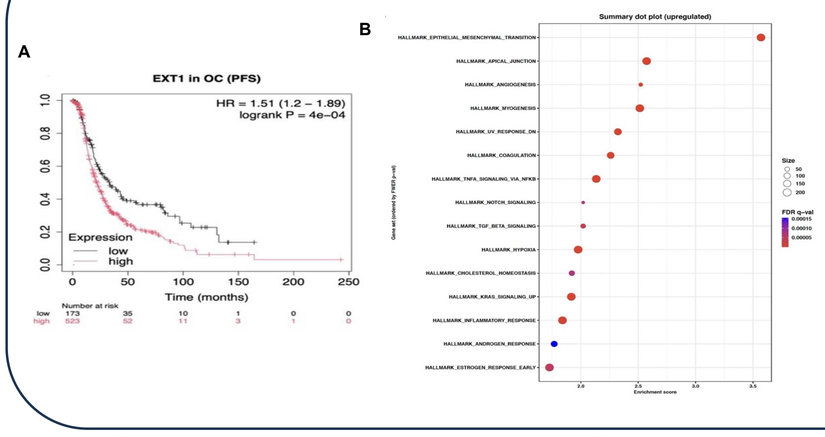
Co-culture: HUVEC cells and PEO1 clones were co-cultured at a 4:1 ratio and seeded on Matrigel. A mixed culture medium (1:1) consisting of Prigrow I medium and RPMI supplemented with 2% FBS was used.

Tube formation assay: HUVEC cells were exposed to conditioned medium derived from PEO1 clones.

ELISA: conditioned medium were collected at 96 hours and analyzed using a specific ELISA kit according to the manufacturer's instructions.

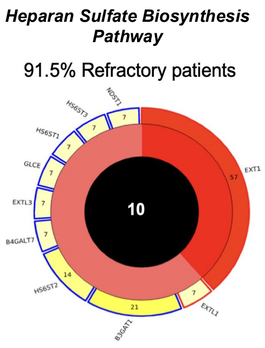
RESULTS & DISCUSSION

EXT1: correlation with survival and biofunctions



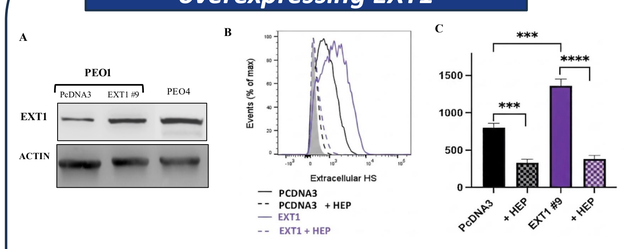
High EXT1 expression is significantly associated with poor prognosis and reduced survival in ovarian cancer patients. Consistent with this clinical phenotype, GSEA analysis reveals a marked enrichment of genes involved in the epithelial-mesenchymal transition (EMT), suggesting that EXT1 promotes tumor aggressiveness by enhancing cellular plasticity and metastatic progression (B).

Alterations in the Heparan Sulfate Biosynthesis Pathway in Refractory TCGA-OC Patients



The heparan sulfate biosynthesis pathway is heavily altered in 91.5% of refractory ovarian cancer patients (TCGA-OC). Within this pathway, EXT1 emerges as the most frequently altered gene (57%), suggesting that EXT1-driven heparan sulfate remodeling plays a pivotal role in the development of drug resistance.

Ovarian cancer cells overexpressing EXT1



Experimental model validation confirmed that the **PEO1 EXT1 #9** clone exhibits a significant increase in EXT1 protein expression compared to the **PcDNA3** control, effectively mimicking the profile observed in the resistant **PEO4** line (A). Flow cytometry analysis (B-C) reveals a marked increase in extracellular heparan sulfate (HS), the specificity of which was validated by the drastic signal reduction following heparinase (**+ HEP**) treatment.

EXT1 modulates tumor secretome

To identify soluble factors potentially involved in the modulation of angiogenesis, we quantified the secretion levels of key angiogenic mediators in conditioned media derived from PEO1 pcDNA3, PEO1 EXT1 #9, and PEO4 cell lines using ELISA assays. This analysis revealed significant variations in the secretion of VEGF, IL-8, and Endothelin-1 following EXT1 overexpression, suggesting a potential role of EXT1 in reshaping the angiogenic secretome. These changes may reflect a broader impact on the tumor microenvironment and highlight differential regulation of pro-angiogenic signalling pathways across the analyzed cell models.

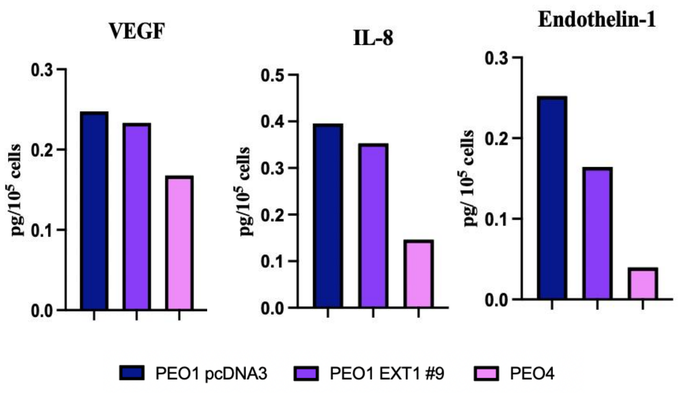
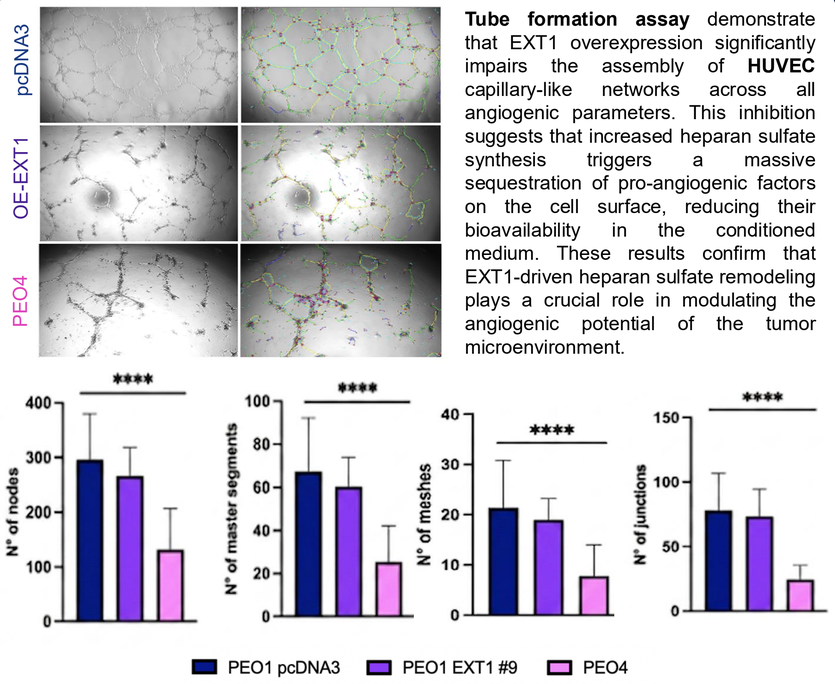


Figure 1. ELISA-based quantification of VEGF, IL-8, and Endothelin-1 in conditioned media collected after 96 hours from PEO1-pcDNA3 (control), PEO1 EXT1 #9, and PEO4 cells. Results are expressed as pg/10^5 cells. The PEO1 EXT1 #9 clone shows lower VEGF levels compared to PEO4 and a reduction in Endothelin-1 compared to the PEO1-pcDNA3 control, while IL-8 levels are intermediate between those observed in the control and in PEO4.

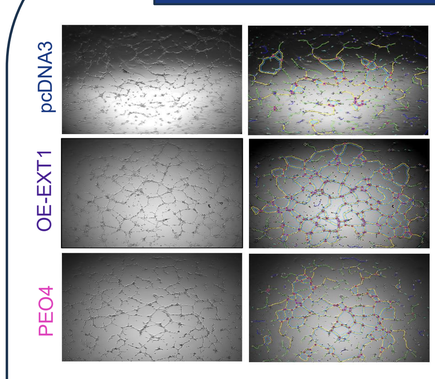
HS-dependent angiogenic signalling



Tube formation assay demonstrate that EXT1 overexpression significantly impairs the assembly of **HUVEC** capillary-like networks across all angiogenic parameters. This inhibition suggests that increased heparan sulfate synthesis triggers a massive sequestration of pro-angiogenic factors on the cell surface, reducing their bioavailability in the conditioned medium. These results confirm that EXT1-driven heparan sulfate remodeling plays a crucial role in modulating the angiogenic potential of the tumor microenvironment.

Figure 2. The conditioned medium modulates the formation of vascular-like structures in HUVECs. Evaluation of endothelial network formation in HUVECs exposed to conditioned media from PEO1 pcDNA3, PEO1 EXT1 #9, and PEO4 cells. The analysis quantifies key angiogenic parameters, including the number of nodes, meshes, junctions, and master segments. Representative phase-contrast images are shown alongside their corresponding skeletonized maps generated via *ImageJ/Angiogenesis Analyzer* software to visualize vascular-like structures.

HS remodeling in tumor–endothelial crosstalk



Direct co-culture assays demonstrate that EXT1 overexpression stimulates the formation of vascular networks in HUVECs, with a significant increase in all angiogenic parameters compared to the control. These results indicate that physical contact and heparan sulfate-mediated paracrine signalling are sufficient to promote endothelial organization. In this context, heparan sulfate acts as a scaffold that facilitates the local presentation of growth factors to HUVEC receptors, enhancing paracrine communication within the tumor microenvironment.

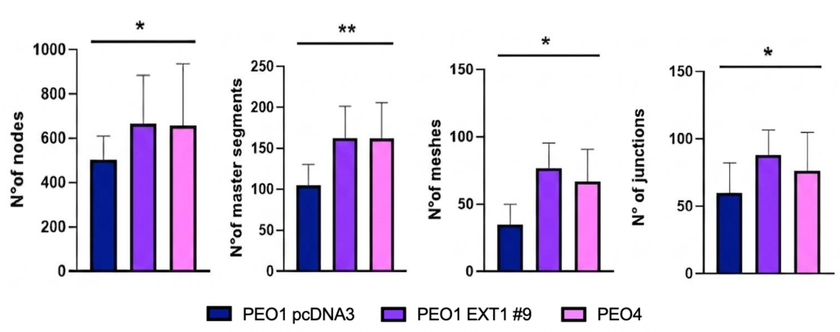
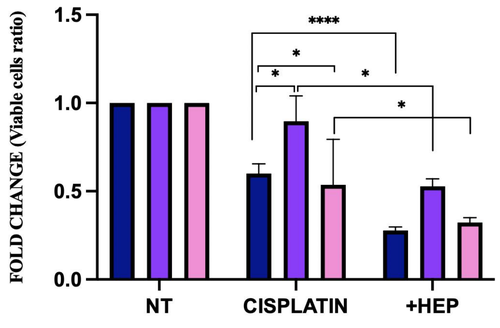


Figure 3. Effect of direct co-culture between ovarian cancer cells and HUVECs on capillary-like network formation. The histograms represent the quantification of morphometric parameters (nodes, segments, meshes, and junctions) obtained using *ImageJ/Angiogenesis Analyzer* software. Representative micrographs show the structural organization of HUVECs (left panels) alongside their corresponding skeletonized analysis maps (right panels).

EXT1 promotes cisplatin resistance

OE-EXT1 #9 cells and the platinum-resistant PEO4 cell line exhibited reduced sensitivity to cisplatin and oxaliplatin compared with pcDNA3 control cells. Heparinase treatment significantly enhanced the cytotoxic effect of both drugs, leading to a marked decrease in cell viability in resistant cells. These findings suggest that heparan sulfate contributes to platinum resistance and that its enzymatic degradation can improve the response to platinum-based chemotherapy.



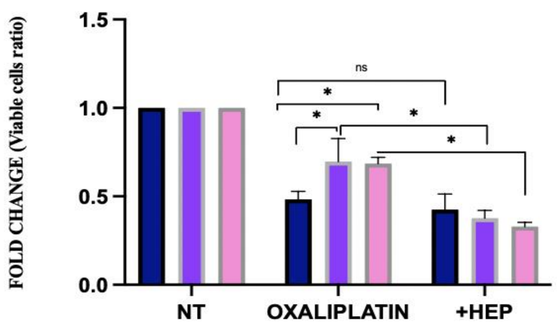


Figure 4. Platinum drug sensitivity. Cell viability was assessed by MTT assay after 72 h treatment with cisplatin (50 μM) (A) or oxaliplatin (50 μM) (B) in pcDNA3, OE-EXT1 #9, and PEO4 cells, in the presence or absence of heparinase (+HEP). EXT1 overexpression and the resistant phenotype of PEO4 cells were associated with increased cell survival following platinum treatment. Enzymatic degradation of heparan sulfate by heparinase enhanced sensitivity to platinum compounds, significantly reducing cell viability.

CONCLUSIONS

EXT1 overexpression influences the angiogenic properties of ovarian cancer cells by altering secretome composition and remodeling the extracellular matrix. Using the longitudinal PEO1/PEO4 model, derived from the same patient before and after the acquisition of chemoresistance, we demonstrated that elevated EXT1 expression regulates growth factor signalling dynamics and contributes to tumor progression. ELISA analyses of conditioned media collected after 96 hours revealed significant alterations in the levels of key angiogenic factors, including VEGF, IL-8, and Endothelin-1. These changes were associated with modified endothelial responses in HUVEC tube formation assays, suggesting that EXT1-dependent heparan sulfate remodeling affects the bioavailability and activity of pro-angiogenic signals. Furthermore, co-culture experiments showed that EXT1-overexpressing cells generate a microenvironment that strongly supports angiogenesis through direct interactions with endothelial cells [3].

In addition to its role in tumor–endothelial crosstalk, EXT1 contributes to the development of chemoresistance. OE-EXT1 #9 and PEO4 cells exhibited reduced sensitivity to cisplatin treatment. However, enzymatic degradation of heparan sulfate by heparinase restored drug responsiveness and significantly decreased cell viability, supporting a direct contribution of heparan sulfate accumulation to platinum resistance.

Collectively, these findings identify EXT1 as a key regulator of both tumor–endothelial interactions and chemotherapy response. Our results suggest that EXT1-driven heparan sulfate remodeling shapes the tumor microenvironment by modulating growth factor availability, endothelial cell behavior, and sensitivity to platinum-based therapies. These observations highlight EXT1 as a promising therapeutic target to limit ovarian cancer progression, plasticity, and treatment resistance.

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